COMPREHENSIVE GENE EXPRESSION CHARACTERIZATION OF THE SWARM RAT CHONDROSARCOMA

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Introduction
Chondrosarcoma is the second most common type of skeletal malignancy. Current treatment protocols are based primarily on surgical resection since its response to chemotherapy and radiotherapy is very poor. Survival rates for grade III tumors are only 29%. A better understanding of its biology is necessary for the design of new therapeutic modalities that could improve survival rates. An in vitro and an animal model will be of great benefit for this purpose.

The Swarm rat chondrosarcoma is a tumor tissue line that has been maintained through the years by serial subcutaneous injections, and closely resembles human chondrosarcomas histologically. These cells have been the subject of extensive biochemical studies and have been used as a source of cartilage extracellular matrix molecules. In addition, injection of the cells on rat tibias resulted on a tumor that resembles the human counterpart (1). These characteristics make it very appealing for studies of chondrosarcoma biology. We have previously reported the initial characterization of the most abundant genes in this tumor (2). This study was designed to further characterize the gene expression profile of the Swarm rat chondrosarcoma, and to make the result available to the research community.

Material and Methods

Swarm rat chondrosarcoma induction: Animal usage was institutionally reviewed and approved. Male Sprague-Dawley rats were used and the tumor induced by subcutaneous cell injection as previously described (1).

RNA isolation: Total RNA was extracted by a modified method of Smale and Sasse (3). Briefly, tissue samples were frozen immediately after excision and the frozen tissue was powdered in a Spex Freezer Mill (SPEX, Metuchen, NJ). A TRIZOL/chloroform extraction was then performed followed by an isopropanol/ high salt precipitation and a DNase I digestion. The sample was then applied to the top of CsTFA working solution (density of 1.51 g/ml in 100 mM EDTA, pH7.0). Centrifugation was performed in a Beckman SW28 rotor at 25,000 rpm for 24 hr at 15°C. After ultracentrifugation, the pellet was dissolved in 400 µl of TE and RNA was precipitated with 1o µl of 1 M acetic acid and 1 µl of 10% sodium acetate buffer chilled at -70°C for 20 min. Poly(A)+ RNA was isolated by chromatography on oligo(dT)-cellulose (New England Biolabs) according to manufacturers’ instructions, except that two rounds of purification were performed.

cDNA library construction: High-quality poly (A)+ RNA isolated was used for construction of the cDNA library. cDNA library construction was essentially as described by Soares et al (3). Briefly, 1 µg poly(A)+ RNA was annealed at 37°C with a twofold mass excess of GCGGCCGC synthesis, we used the oligonucleotide 5'-AACTGGGAAGAATTC and thus serves as an identifier. As a primer for first-strand cDNA sequence is a sequence of 10 nucleotides that is unique for each library construction was essentially as described by Soares et al (3). Briefly, 1 µg of cDNA was used for construction of the cDNA library. cDNA library construction:

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cDNA library construction: High-quality poly (A)+ RNA isolated was used for construction of the cDNA library. cDNA library construction was essentially as described by Soares et al (3). Briefly, 1 µg poly(A)+ RNA was annealed at 37°C with a twofold mass excess of 5'-AAGGCCTGAAAGTTCCGGCGCCCGCNGNNNNNNN(T)18-3', which contains a Not I site (underline) and a tag sequence (N10), specific for each library. Double-stranded cDNAs were sized-selected by gel filtration over a long (64 cm) and narrow (0.2 cm diameter) Bio-Gel A 50- to 1000-fold molar excess of adapters. After ligation to EcoR I adaptors, the cDNAs were digested with Not I and cloned directionally into the EcoRI and Not I sites of the pT7T3-Pac vector. Library normalization was performed as previously described by Bonaldo et al (4).

DNA sequencing and Data analysis: Double-stranded plasmid DNA templates were prepared using the 96-well microwave protocol (1) and sequenced (2) from the 3' end using Rhodamine dye terminator chemistry (Applied Biosystems) with universal forward and reverse M13 primers. Sequencing reactions were assembled with a Robbins ScientificHydra-96 Microdispenser and then transferred to a MJ Research PTC-222 Peltier thermal cycler for cycle sequencing. Reaction products were ethanol precipitated, resuspended in formamide, and electrophoresed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). Nucleic acid database searches were performed using the BLAST family of programs.

Results

In order to analyze the gene expression profile of the Swarm rat chondrosarcoma, approximately 6,874 random ESTs were sequenced from the 3' end. To aid in the analysis of expressed transcripts, overlapping ESTs corresponding to the same gene were grouped into clusters. A representative member from each cluster was then compared against public gene databases. A variety of protein families are represented including extracellular matrix proteins, growth factors, transcription factors, oncogenes and tumor suppressor genes, membrane receptors, matrix degrading enzymes, etc. These data suggest that the Swarm rat chondrosarcoma is a very active and metabolically dynamic tissue, and there are a large number of expressed transcripts that have not been previously described. The presence of collagen type II, aggrecan, cartilage oligomeric protein (COMP), fibromodulin, and others, supports the chondrocytic nature of this tumor and the authenticity of the tissue used for the preparation of the library.

Discussion

This study describes the use of large-scale EST sequencing approach to identify genes that are expressed in Swarm rat chondrosarcoma. Until recently, efforts to identify genetic molecular changes relied on laborious, and necessarily limited, one gene-at-a-time approach. Large-scale EST sequencing offers a powerful tool for investigating the gene expression pattern of a large number of genes simultaneously, allowing the analysis of complex biologic systems rather than single aspects of them. Analysis of the expressed ESTs revealed a complex pattern of gene expression, including many genes not yet reported to be expressed by chondrocytes.

Many cartilage matrix components were found including collagen II and aggrecan, but there was minimal expression of collagen X and XI. These two molecules are associated with specific features of differentiation in chondrocytes, and those aspects may be altered in chondrosarcoma cells. Interestingly, there was no evidence of expression of lysyl oxidase, and LOH or mutations on lysyl oxidase have been implicated in tumor suppression and cell growth regulation in several tumor models. Several proto-oncogenes and oncogenes-associated were also expressed, as it was vimentin. High expression of vimentin has been observed in high-grade prostate tumors; however, its significance in chondrosarcoma is not known.

Future investigations will take advantage of microarray hybridization technology and SAGE to capture differential expression of hundreds of other mRNAs toward a complete description of the molecular expression profiles of Swarm rat chondrosarcoma. Ultimately, these studies will help to unravel the molecular pathways and networks that are altered in this animal model. We anticipate that this information will lead to a deeper understanding of the biology of chondrosarcoma, and might allow the development of more specific and targeted therapies.

References